

Short communication

Self-compatibility in *Chionochloa pallens* and *C. macra* (Poaceae)
confirmed by hand pollination of excised styles

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Abstract In *Chionochloa pallens* and *C. macra* (Poaceae), pollen tube growth on excised styles planted on agar showed that self-pollen was as viable as cross-pollen. While selfing is therefore possible, other evidence (including pollen-ovule ratios) suggests it is not the rule in *Chionochloa*.

Keywords *Chionochloa*; Poaceae; pollen tube growth; self-compatibility; self-pollination

INTRODUCTION

Chionochloa (Poaceae) is a genus of 22 species of large tussock grasses which dominate much of the subalpine zone in the South Island of New Zealand (Connor 1991). The species are wind pollinated and also vary widely in their flowering effort from year to year, i.e., are mast seeding (Connor 1991; Kelly et al. 1992). One possible reason advanced for mast seeding is that wind pollination may be more efficient at high flowering intensities (Kelly 1994).

However, this can only be a selective benefit if the species are outcrossing. Where a species is totally or largely self-pollinating, the abundance of flowers on adjacent plants would be largely irrelevant to its reproductive success.

Connor (1960, 1967) demonstrated self-compatibility in six *Chionochloa* species (*C. cheesmanii*, *C. conspicua*, *C. flavescens*, *C. rigida*, and *C. rubra*) on two replicate plants per species by enclosing culms inside plastic bags and measuring set seed. Here we extended Connor's work by investigating two more species (*C. pallens* and *C. macra*) and by using the method of Lundqvist (1961) to look directly at the ability of self-pollen tubes to reach the ovule after fertilisation. We also consider other evidence for outcrossing in the genus.

METHODS

Flowering shoots were obtained from three *C. macra* plants cultivated in the gardens of Landcare Research, Lincoln, in December 1995, and from 18 *C. pallens* plants at 1070 m altitude on Mt Hutt, central Canterbury (study site described in Kelly et al. 1992 and Kelly & Sullivan 1997) in early January 1996. Shoots were returned to the lab on the day of collection and kept in still air with their bases in water; pollinations were performed within 1–2 days of collection.

To determine whether the species are self-compatible, we used Lundqvist's (1961) method of hand-pollinating pistils that have been removed from the plant and placed in a growth medium. Pistils were taken from florets that were near anthesis, as judged by the dark colour and mature appearance of the anthers in the florets and by the orderly progression of flowering within a spikelet. The pistils were "planted" into petri dishes containing the agar medium of Lundqvist (1961) and incubated overnight at room temperature. Either self- or cross-pollen was taken from inflorescences that flowered in the lab, and brushed onto a stigma in the petri dishes by means of a human eyelash. Pollinations took place



Fig. 1 UV-fluorescence micrograph of the style of a *Chionochloa pallens* pistil showing normal pollen tube growth following self-pollination of the excised pistil in agar growth medium.

approximately 16 hours after pistils were placed in the agar medium. Four hours after pollination, pistils were fixed in 3:1 ethanol:acetic acid for three hours, cleared in 10 N NaOH for 30–90 minutes, rinsed with water, stained with aniline blue, and mounted in dilute glycerine. Longer clearing times (60–90 minutes) allowed the best views of pollen tubes in the ovary, although the stigmas tended to fragment. Pollen germination and pollen tube development in the pistils were examined by fluorescence microscopy. We particularly looked for signs of the self-incompatibility reaction typical of grasses (see Heslop-Harrison 1982). Stigmas were also examined for signs of physical damage (broken hairs) or senescence (brown staining). On each *C. pallens* petri dish, an unpollinated pistil was left in the centre to check that there was no contamination from airborne pollen. These control pistils were processed and examined as above but no pollen was seen on them.

Self-compatibility was deemed to be shown for an individual plant if its own pollen gave good pollen tube growth into the ovary in at least one selfed pistil. Where an individual plant's pollen from a floret did not produce tubes on either selfed or crossed stigmas, the pollen from that floret was deemed to be inviable.

RESULTS

Viable pollen seemed to have a very short life after anthesis and initially some pollinations produced no pollen tube growth on any pistil, selfed or crossed. Closer attention to using pollen immediately after anthesis gave more reliable germination on the excised stigmas, with abundant pollen tubes often visible down the stigma and into the ovary (Fig. 1). Anthesis usually occurred between 10 am and noon, and flowering shoots were checked every 30 minutes so pollen could be used soon after anthesis.

For *C. macra*, two of the three plants tested gave good pollen growth under self- and cross-pollination. The pollen of the third plant failed to germinate on any pistil. For *C. pallens*, 14 of the 18 plants showed strong pollen tube growth under self-pollination. One plant had no pollen to use for testing, and two had no viable pollen. A single plant gave pollen tube growth on crossed stigmas, but not on two selfed pistils; however, these selfed pistils were both brown and may have been too old.

There was no sign on the stigmas of twisted, callose-filled pollen tubes which might indicate abnormal growth due to self-incompatibility (Heslop-Harrison 1982). The number of pollen tubes reaching

into the style and into the ovary was counted in every case where some pollen tube formation was visible. There was no indication in styles or ovaries of greater numbers of pollen tubes with cross-pollen than with self-pollen (Table 1), with differences being non-significant and usually favouring self-pollen. This shows that self-pollen was capable of frequently reaching the ovary.

DISCUSSION

In every case where viable pollen was tested on seemingly healthy pistils in both *C. macra* and *C. pallens*, self-compatibility was found. The pollen tubes showed none of the signs of self-incompatibility such as abnormal growth, excessive callose deposition, or reduced penetration into the ovary (Table 1). This confirms the general conclusions of Connor (1967) for six other species of *Chionochloa*, that self-compatibility is common in the genus. To fully show self-compatibility would require hand pollination with known pollen, followed by raising healthy plants from the resulting seed. However, controlled hand pollinations on intact grasses are very difficult. Our method showed healthy pollen tube growth with self-pollen; taken in conjunction with Connor's work on seed produced inside bagged culms, a strong case is made for self-compatibility.

Connor's work was based on two plants per species, and we studied only three plants for *C. macra*. However, the information for *C. pallens* gives no indication of plant-to-plant variation in self-compatibility, with good self-pollen tube growth in all 14 cases where satisfactory material was available.

Of course, the fact that self-pollination is possible in *Chionochloa* does not mean that the rate of self-pollination is high in natural populations. Wild rice (*Oryza perennis*) is a self-compatible grass, but

different strains span the range from nearly complete self-pollination to commonly outcrossing (Oka & Morishima 1967). When self and outcross pollen compete in the same pistil, the self-pollen may be at a disadvantage ("cryptic self-incompatibility"; see Jones 1994). Post-pollination events may also favour outcrossed seeds, e.g., selfed fruits may be aborted more frequently than outcrossed fruits (Becerra & Lloyd 1992). Significant inbreeding depression at any stage in the life cycle may produce a new generation that is predominately outcrossed, even if self-pollination is common. Connor (1960, 1967) showed that apparently normal seed was produced by bagged inflorescences in six *Chionochloa* species and that plants grown from this seed were not significantly smaller or less fecund than comparable outcrossed plants. However, there is an indication in Connor's (1967) work of possible reduced survival of seedlings: "when the [seedlings] were to be planted out over a year later, there had been many losses, especially among the inbred families.... in *C. cunninghamii cunninghamii* the few S1 [selfed] survivors were just as vigorous as those from open-pollinated seed, but the level of S1 loss was about six times that of OP [open-pollinated] plants". Therefore it is possible that selfed offspring in *C. pallens* and/or *C. macra* might show some inbreeding depression. We did not test for this.

Although there are no direct measurements of the outcrossing rate (by electrophoresis, for example) of any *Chionochloa* population, there are indications that outcrossing may be frequent. The florets open widely at anthesis to expose both stigmas and anthers (personal observation) which would allow outcrossing in the typically dense populations of most *Chionochloa* species. The relatively large anthers of *Chionochloa* species also suggest an outcrossing breeding system. The pollen-ovule ratio is a reliable predictor of average selfing rate in diverse angiosperms (Cruden 1977; Preston 1986; Robertson & Lloyd 1991); since grasses are uniovulate, the pollen-ovule ratio is strongly correlated with anther size. *Chionochloa pallens* anthers are relatively large, with an average length of 3.83 mm (McKone 1990). This is comparable with the 4.02 mm anthers in the obligately outcrossing grass *Bromus inermis*; partially selfing *Bromus* species have anthers that average from 1.5 to 1.9 mm, and the selfing species *B. tectorum* has anthers that are only 0.65 mm long (McKone 1987).

There is other indirect evidence of regular outcrossing in *Chionochloa*. The possibility of inbreeding depression (see above) suggests that the species

Table 1 Mean numbers of pollen tubes reaching into the style or ovary of *Chionochloa* spp. pistils in agar culture (number of pistils in parentheses); results of two-sample t-tests are also shown.

	<i>C. macra</i>		<i>C. pallens</i>	
	style	ovary	style	ovary
Self	4.33 (6)	4.25 (4)	6.08 (25)	4.56 (25)
Cross	4.38 (8)	3.00 (5)	4.68 (19)	3.19 (16)
t	0.04	-1.03	-1.77	-1.89
P =	0.97	0.34	0.08	0.07

are normally outcrossing (Schemske & Lande 1985). Also, natural hybrids between a number of *Chionochloa* species are found in the field (Connor 1991). Finally, indications that pollination is reduced in *C. pallens* at lower flowering densities (Kelly & Sullivan 1997) suggest that not all pollination comes from pollen on the same inflorescence.

Therefore, our data support the conclusion that *Chionochloa* species are generally capable of self-pollination, as is common in the New Zealand flora (Webb & Kelly 1993). However, at least some outcrossing is likely. This has implications for a number of fields of study, including mast seeding, where benefits by more effective pollination in mast years are at least possible.

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